

AMENDMENT AND RESPONSE UNDER 37 CFR § 1.111
Serial Number: 09/464158
Filing Date: December 16, 1999
Title: ASSAY FOR CARBOHYDRATE-FREE TRANSFERRIN

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IN THE CLAIMS

Please amend the claims as follows:

1. (Currently Amended) A method for ~~the determination of carbohydrate-free transferrin in a body fluid for use in~~ the assessment of elevated alcohol consumption, said method comprising
 - (a) contacting a sample of ~~said a~~ body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand;
 - (b) separating a carbohydrate-free transferrin containing fraction not binding to said ligand and contacting the separated fraction with an anti-transferrin antibody or an anti-transferrin antibody fragment; and
 - (c) ~~determining~~ detecting the content of carbohydrate-free transferrin in said fraction and thereby determining the content of carbohydrate-free transferrin in said sample, wherein said content of carbohydrate free transferrin is used in the assessment of elevated alcohol consumption.
2. (Original) A method as claimed in claim 1, wherein the sample is blood or obtained from blood.
3. (Previously Presented) The method as claimed in claim 1, wherein the carbohydrate-binding ligand is selected from the group consisting of antibodies, antibody fragments, lectins, mammalian carbohydrate-binding proteins, microbial carbohydrate-binding proteins, and mixtures thereof.
4. (Previously Presented) The method as claimed in claim 1, wherein in step (a) a panel of more than one type of lectin is used as a carbohydrate binding ligand.
5. (Previously Presented) The method as claimed in claim 1, wherein the carbohydrate-binding ligand is selected from the group consisting of *Sambucus nigra* lectin, *Sambucus sielbodiana* lectin, wheatgerm agglutinin, *Maackia amurensis* lectin, *E. coli* K99 lectin, *Helicobacter pylori* lectin, *Ricinus communis* lectin, *Crotalaria junctae* lectin, anti-sialic acid antibodies, and mixtures thereof.

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6. (Previously Presented) The method as claimed in claim 1, wherein the separation step (b) is by precipitation, centrifugation, filtration or chromatographic methods.
7. (Previously Presented) The method as claimed in claim 1, wherein the carbohydrate-binding ligand is immobilized.
8. (Previously Presented) The method as claimed in claim 1, wherein an ion exchange step to remove or deplete carbohydrate-carrying transferrins in the sample is performed prior to step (a).
9. (Previously Presented) The method as claimed in claim 1, wherein determining the transferrin content in step (c) is achieved by turbidometric or nephelometric means.
10. (Currently Amended) A kit for use in a method as defined in claim 1, said kit comprising:
 - one or more carbohydrate-binding ligands;
 - means for separating unbound carbohydrate-free transferrin from ligand-bound carbohydrate-containing transferrin; and
 - means for ~~determining~~ detecting the carbohydrate-free transferrin content in the separated portion which determines the content of carbohydrate-free transferrin in the sample.
11. (Previously Presented) The kit as claimed in claim 10, wherein said means for determining the carbohydrate-free transferrin content comprises an anti-transferrin antibody or an anti-transferrin antibody fragment; and optionally an opacification enhancer.
12. (Previously Presented) The kit as claimed in claim 10, further comprising a carbohydrate-free transferrin solution of known concentration or a set of such solutions having a range of carbohydrate-free transferrin concentrations.
13. (Withdrawn) A method for the detecting carbohydrate-free transferrin in a body fluid for use as an indicator of alcohol abuse, said method comprising

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- (a) contacting a sample of said body fluid with an immobilized carbohydrate-binding ligand to bind any carbohydrate-containing moieties in the sample to the immobilized ligand;
- (b) separating any unbound carbohydrate-free transferrin from any bound carbohydrate-containing moieties;
- (c) contacting any separated carbohydrate-free transferrin with an anti-transferrin antibody or an anti-transferrin antibody fragment to form a conjugate; and
- (d) detecting the presence of any carbohydrate-free transferrin anti-transferrin antibody conjugate by turbidometry or nephelometry.

14. (Withdrawn) The method of claim 13, wherein the presence of any carbohydrate-free transferrin is indicative of alcohol abuse.

15. (Withdrawn) The method of claim 13, wherein the method is free from the influence of amino acid sequence polymorphism in the polypeptide backbone of an abuser's transferrin.

16. (Withdrawn) The method of claim 13, wherein the method is independent of the abuser's race.

17. (Withdrawn) A kit for use in a method of claim 13, the kit comprising:

- one or more carbohydrate-binding ligands;
- means for separating unbound carbohydrate-free transferrin from bound carbohydrate-containing transferrin; and
- means for detecting any carbohydrate-free transferrin.

18. (New) A method for the assessment of elevated alcohol consumption, comprising

- (a) contacting a sample of a body fluid with a carbohydrate-binding ligand to bind carbohydrate and carbohydrate-containing moieties in the sample to the ligand;
- (b) separating a carbohydrate-free transferrin containing fraction not binding to the ligand from the ligand and contacting the separated fraction with an anti-transferrin antibody or an anti-transferrin antibody fragment, wherein at least 60% of transferrin molecules in the carbohydrate-free transferrin containing fraction are carbohydrate-free transferrin molecules; and

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(c) detecting a presence or amount of carbohydrate-free transferrin molecules in the fraction, wherein the presence or amount of carbohydrate free transferrin molecules is indicative of elevated alcohol consumption.

19. (New) The method as claimed in claim 18, wherein at least 70% of the transferrin molecules in the carbohydrate-free transferrin containing fraction are carbohydrate-free transferrin molecules.

20. (New) The method as claimed in claim 19, wherein at least 80% of the transferrin molecules in the carbohydrate-free transferrin containing fraction are carbohydrate-free transferrin molecules.

21. (New) The method as claimed in claim 20, wherein at least 90% of the transferrin molecules in the carbohydrate-free transferrin containing fraction are carbohydrate-free transferrin molecules.

22. (New) The method as claimed in claim 21, wherein at least 95% of the transferrin molecules in the carbohydrate-free transferrin containing fraction are carbohydrate-free transferrin molecules.

23. (New) The method as claimed in claim 1, wherein a presence of carbohydrate free transferrin in the fraction is indicative of elevated alcohol consumption.